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Phytoremediation of heavy metals: A strategy for the removal of toxic metals from the environment using plants

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Abstract

Heavy metals constitute a heterogeneous group of elements; a relatively high density of approximately 6 g cm^{-3} is their common characteristic with atomic weight more than that of iron (Alloway, 1997) [3]. Sources of heavy metal contaminants in soils include metaliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries (Alloway 1995) [2]. Toxic heavy metals cause DNA damage, and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Knasmuller *et al.*, 1998; Baudouin *et al.*, 2002) [46, 8]. Metal-contaminated soil can be remediated by chemical, physical or biological techniques (McEldowney *et al.*, 1993) [56]. Chemical and physical treatments irreversibly affect soil properties, destroy biodiversity and may render the soil useless as a medium for plant growth. Phytoremediation involves the use of plants to remove, transfer, stabilize and/or degrade contaminants in soil, sediment and water (Hughes *et al.*, 1997) [43]. This plant based technology has gained acceptance in the past ten years as a cheap, efficient and environment friendly technology especially for removing toxic metals. Plant based technologies for metal decontamination are extraction, volatilization, stabilization and rhizofiltration. Various soil and plant factors such as soil's physical and chemical properties, plant and microbial exudates, metal bioavailability, plant's ability to uptake, accumulate, translocate, sequester and detoxify metal amounts for phytoremediation efficiency. Use of transgenic to enhance phytoremediation potential seems promising. Despite several advantages, phytoremediation has not yet become a commercially available technology. Progress in the field is hindered by lack of understanding of complex interactions in the rhizosphere and plant based mechanisms which allow metal translocation and accumulation in plants.

Keywords: Phytoremediation, heavy metals, strategy, removal, toxic metals, environment

Introduction

Heavy metals constitute a heterogeneous group of elements; a relatively high density of approximately 6 g cm^{-3} is their common characteristic with atomic weight more than that of iron (Alloway, 1990) [3]. Over recent decades, the annual worldwide release of heavy metals reached 22,000 t (metric ton) for cadmium, 939,000 t for copper, 783,000 t for lead and 1,350,000 t for zinc (Singh *et al.*, 2003) [79]. Sources of heavy metal contaminants in soils include metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries (Alloway., 1995) [2]. Ground-transportation also causes metal contamination. Highway traffic, maintenance, and de-icing operations generate continuous surface and ground- water contaminant sources. Tread ware, brake abrasion, and corrosion are well documented heavy metal sources associated with highway traffic (Ho and Tai 1988; Fatoki 1996; García and Millán 1998; Sánchez Martínet *et al.*, 2000) [42, 33, 35, 76].

The ability of plant to germinate and establish under heavy metal stress is an early indication of tolerance of the plant. Seed germination was the first physiological process affected by heavy metals when present in the culture medium (Peralta *et al.*, 2001) [36]. Decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Breckle, 1991). Shoot growth is an important morphological parameter related to growth and development of whole plant which is severely affected by Cr and other heavy metals (Khan *et al.*, 2000; Rout *et al.*, 1997) [45].

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Toxic heavy metals cause DNA damage, and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Knasmuller *et al.*, 1998; Baudouin *et al.*, 2002) [46, 8]. Exposure to high levels of these metals has been linked to adverse effects on human health and wildlife. Lead poisoning in children causes neurological damage leading to reduced intelligence, loss of short term memory, learning disabilities and coordination problems. The effects of arsenic include cardiovascular problems, skin cancer and other skin effects, peripheral neuropathy (WHO 1997) and kidney damage. Cadmium accumulates in the kidney and is implicated in a range of kidney diseases (WHO 1997) [93]. The principal health risks associated with mercury are damage to the nervous system, with such symptoms as uncontrollable shaking, muscle wasting, Partial blindness, and deformities in children exposed in the womb (WHO 1997) [93].

Metal-contaminated soil can be remediated by chemical, Physical or biological techniques (Mc Eldowney *et al.*, 1993) [56]. Chemical and physical treatments irreversibly affect soil properties, destroy biodiversity and may render the soil useless as a medium for plant growth. These remediation methods can be costly. Table 1 summarizes the cost of different remediation technologies. Among the listed remediation technologies, Phyto extraction is one of the lowest cost techniques for contaminated soil remediation. There is a need to develop suitable cost-effective biological soil remediation techniques to remove contaminants without affecting soil fertility. Phytoremediation could provide sustainable techniques for metal remediation.

The term phytoremediation (“Phyto” meaning plant, and the Latin suffix “remedium” meaning to clean or restore) refers to a diverse collection of plant based technologies that use either naturally occurring, or genetically engineered, plants to clean contaminated environments (Cunningham *et al.*, 1997; Flathman and Lanza 1998) [19, 34]. Phytoremediation involves the use of plants to remove, transfer, stabilize and/or degrade contaminants in soil, sediment and water (Hughes *et al.*, 1997) [43]. Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of indigenous metals in their tissues without symptoms of toxicity (Reeves and Brooks 1983; Baker and Brooks 1989; Baker *et al.*, 1991; Entry *et al.*, 1999) [4, 6, 65, 32]. The idea of using plants to extract metals from contaminated soil was reintroduced and developed by (Utsunomyia., 1980) [88] and Chaney (1983) [16]. The first field trial on Zn and Cd phyto extraction was conducted by (Baker *et al.*, 1991) [4].

Table 1: Cost of different remediation technologies (Glass 1999) [38]

Process	Cost (US\$/ton)	Other factors
Verification	75–425	Long-term monitoring
Land filling	100–500	Transport/excavation/ Monitoring
Chemical Treatment	100–500	Recycling of contaminants
Electro kinetics	20–200	Monitoring
Phytoextraction	5–40	Disposal of phyto mass

Categories of Phytoremediation

Depending on the contaminants, the site conditions, the level of clean-up required, and the types of plants, phytoremediation technology can be used for containment (phyto immobilization and phyto stabilization) or removal (phyto extraction and phyto volatilization) purposes (Thangavel and Subharam 2004) [84]. The four different plant-

based technologies of phyto remediation, each having a different mechanism of action for remediating metal-polluted soil, sediment, or water:

- (1) Phyto stabilization, where plants stabilize, rather than remove contaminants by plant roots metal retention;
- (2) phyto filtration, involving plants to clean various aquatic environments;
- (3) phyto volatilization, utilizing plants to extract certain metals from soil and then release them into the atmosphere by volatilization;
- and (4) phyto extraction, in which plants absorb metals from soil and translocate them to harvestable shoots where they accumulate.

Phytostabilization

Phytostabilization uses certain plant species to immobilize contaminants in soil, through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone and physical stabilization of soils. This process reduces the mobility of contaminants and prevents migration to groundwater or air. This can re-establish vegetative cover at sites where natural vegetation is lacking due to high metal concentrations (Tordoff *et al.*, 2000) [85]. Metal-tolerant species may be used to restore vegetation to such sites, thereby decreasing the potential migration of contaminants through wind, transport of exposed surface soils, leaching of soil and contamination of groundwater (Stoltz and Greger., 2002) [81]. Unlike other phytoremediative techniques, phyto stabilization is not intended to remove metal contaminants from a site, but rather to stabilize them by accumulation in roots or precipitation within root zones, reducing the risk to human health and the environment. For phyto stabilization of metals a combination of trees and grasses work best. Fast-transpiring trees such as ‘Poplar’ maintain an upward flow to prevent downward leaching, while grasses prevent wind erosion and lateral runoff with thin dense root system. Further, grasses do not accumulate as much metals in their shoots as dicot species, minimizing exposure of wildlife to toxic elements (Pilon Smits., 2005) [61]. Phytostabilization is most effective for fine-textured soils with high organic-matter content, but it is suitable for treating a wide range of sites where large areas are subject to surface contamination (Cunningham *et al.*, 1997; Berti and Cunningham 2000) [19, 9]. However, some highly contaminated sites are not suitable for phyto stabilization, because plant growth and survival is impossible (Berti and Cunningham 2000) [9]. Phyto stabilization has advantages over other soil-remediation practices in that it is less expensive, easier to implement, and preferable aesthetically. (Berti and Cunningham 2000; Schnoor 2000) [9, 76].

Phytofiltration

Phytofiltration is the use of plant roots (rhizofiltration) or seedlings (blastofiltration) to absorb or adsorb pollutants, mainly metals, from water and aqueous waste streams (Prasad and Freitas., 2003) [64]. Plant roots or seedlings grown in aerated water absorb, precipitate and concentrate toxic metals from polluted effluents (Dushenkov and Kapulnik 2000; Elless *et al.*, 2005) [25, 30]. Mechanisms involved in bio sorption include chemisorption, complexation, ion exchange, micro precipitation, hydroxide condensation onto the bio surface, and surface adsorption (Gardea-Torresdey *et al.*, 2004) [36]. Rhizofiltration uses terrestrial plants instead of aquatic plants because the former feature much larger fibrous root systems covered with root hairs with extremely large surface areas. Metal pollutants in industrial-process water and in groundwater are most commonly removed by

precipitation or flocculation, followed by sedimentation and disposal of there sulging sludge (Ensley 2000) [31]. The process in volvesraising plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov *et al.*, 1995; Salt *et al.*, 1995; Flathmanand Lanza 1998; Zhu *et al.*, 1999) [25, 73, 34, 96]. Root exudates and changes in rhizosphere pH may also cause metals toprecipitate onto root surfaces. As they become saturated with the metal contaminants, roots or who leplants are harvested for disposal (Flathman and Lanza1998; Zhu *et al.*, 1999) [34, 96]. Several aquatic species have the ability to remove heavy metals from water, including water hyacinth (*Eichhornia crassipes*, Kay *et al.*, 1984; Zhu *et al.*, 1999) [44, 96], pennywort (*Hydrocotyle umbellata* L., Dierberg *et al.*, 1987) [24], and duckweed (*Lemna minor* L., Mo *et al.*, 1989). However, these plants have limited potentialfor rhizofiltration because they are not efficient in removing metals as a result of their small, slow growing roots (Dushenkov *et al.*, 1995) [28]. The high water content of aquatic plants complicates their drying, composting, or incineration. In spite of limitations, Zhu *et al.*, (1999) [96] indicated that water hyacinth is effective in removing trace elements in waste streams. Sunflower (*Helianthus annus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrialcandi dates for removing metals from water. The roots of Indian mustard are effective in capturing Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov *et al.*, 1995) [28], whereas sun flower removes Pb (Dushenkov *et al.*, 1995) [28], U(Dushenkov *et al.*, 1997a), 137Cs, and 90Sr (Dushenkov *et al.*, 1997b) [26, 27] from hydroponic solutions. A novel phyto filtration technology has been proposed bySekharet *al.*, (2004) for removal and recovery of lead (Pb) from wastewaters. This technology uses plant based biomaterial from the bark of the plant commonly called Indian sarsaparilla (*Hemidesmus indicus*). The target of their research was polluted surface water and groundwater at industrially contaminated sites.

Phytovolatilization

Phytovolatilization is the release of pollutants from the plant to the atmosphere as a gas. Although it works well for organics, this can be used for a few inorganics that can exist in volatile form i.e. Se, Hg and As (Hansen *et al.*, 1998; Rugh *et al.*, 1996) [40, 69]. Members of the Brassica genus and some microorganisms are particularly good volatilizes of Se (Terry *et al.*, 1992) [81]. Among the aquatic species, rice, rabbit foot grass, Azolla and pickle weed are the best Se volatilizes (Hansen *et al.*, 1998; Lin *et al.*, 2000; Pilon-Smits *et al.*, 1999; Zayad *et al.*, 2000) [40, 95, 62]. Volatilization of Se involves assimilation of inorganic Seinto the organic sele no amino acids selenocysteine (Se Cys) and selenomethionine (Se Met). The latter can be methylated to for mdimethylselenide (DMSe), which is volatile (Terry *et al.*, 2000) [95].Volatilization of As and Hg has been demonstrated for microorganisms, but these elements do not appear to bevolatilized to significant levels by nontransgenic plants (Rugh *et al.*, 1996) [69]. In Hg-contaminated soils and sediments, microbial activity converts the highly toxic Hg (II) into organomercurials and, under optimum conditions, elemental Hg (which is far lesstoxic) enters the global biogeochemical cycle upon volatilization (Bizily *et al.*, 2000)

[11]. Because volatilization completely removes the pollutant from the site as a gas, without need for plant harvesting and disposal, this is an attractive technology. A risk assessment study for volatile Se and Hg reported that the pollutant was dispersed and diluted to such an extent that it did not pose athreat (Lin *et al.*, 2000; Meagher *et al.*, 2000) [95, 11]. Although phytovolatilization is a passive process, it may be maximized by using plant species with high transpiration rates, by overexpression of enzymes such as cystathionine-V-synthase that mediates S/Se volatilization (Van Huysen *et al.*, 2003) [88] and by transferring gene for Se volatilization from hyper accumulators to non-accumulators (Le Duc *et al.*, 2004) [48].

Phytoextraction

Metal phytoextraction relies on metal-accumulating plants to transport and concentrate polluting metals from soil into the harvestable aboveground shoots (Salt *et al.*, 1998; Vassil *et al.*, 1998) [74, 89]. The plant material can subsequently be used for nonfood purposes (e.g. wood, cardboard) or ashed, followed by disposal in a landfill or, in the case of valuable metals, the accumulated element can be recycled. The latter is termed phyto mining (Chaney *et al.*, 2000) [18]. Popular species for phytoextraction are Indian mustard and sunflower because of their fast growth, high biomass, and high tolerance and accumulation of metals and other inorganics (Blaylock and Huang, 2000; Salt *et al.*, 1995b) [73, 12].

A glasshouse investigation was undertaken to evaluate the natural potential of fenugreek (*Trigonella foenumgraecum* L.), spinach (*Spinacia oleracea* L.), and raya (*Brassica campestris* L.) for cleanup of chromium (Cr)-contaminated silty loam and sandy soils. Figure 1 and 2 summarized the Cr uptake in fenugreek, spinach, and raya increased with increasing level of added Cr in both soils. The uptake of Cr in both shoot and root was highest in raya, followed by spinach and fenugreek. The overall mean uptake of Cr in shoot was almost four times and in root was abouttwo times higher in raya compared to fenugreek. The findings indicated that family *Cruciferae* (raya) was most tolerant to Cr toxicity, followed by *chenopodiaceous* (spinach) and *Leguminosae* (fenugreek). Because raya removed the highest amount of Cr from soil, it could be used for pytoremediation of mildly Cr-contaminated soils. (Dheri, G.S., Brar, M.S. and Malhi, S. 2007) [22].

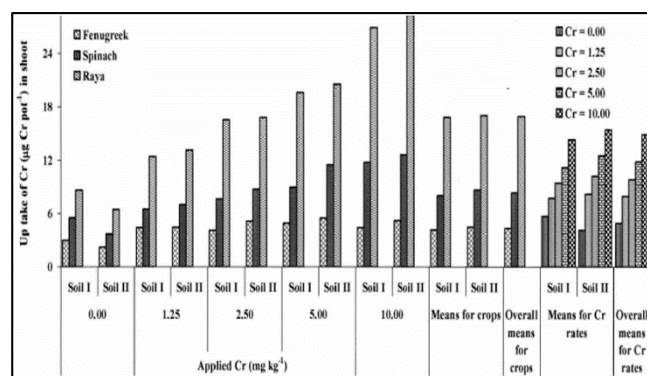


Fig 1: Chromium uptake in shoots of different crops grown in two Cr contaminated soils (soil 1 texture- silty loam and soil 2 – sandy soils)

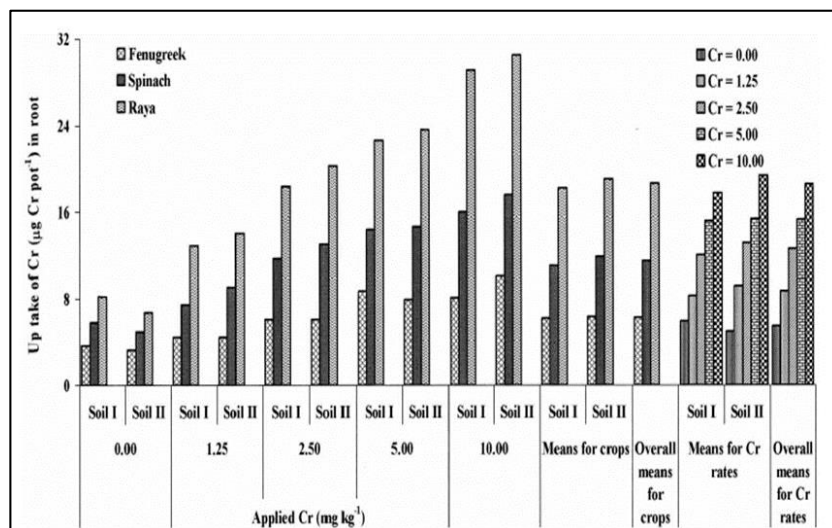


Fig 2: Chromium uptake in the roots of different crops grown in two Cr contaminated soils (soil 1 texture- silty loam and soil 2 – sandy soils)

Deepali and Gangwar 2009 [21] found in their study that the Cr accumulation in the roots and shoots of *Spinach oleracea* in percent are shown fig 3 were higher at minimum concentration. Similarly, Verma *et al.*, (2005) observed that metal take up by water hyacinth (*Eichornia crassipes*) was

higher at low concentration (20%) and decreased thereafter with increase in concentration. From the above result it is also concluded that metal accumulation is higher in roots as compare to shoots.

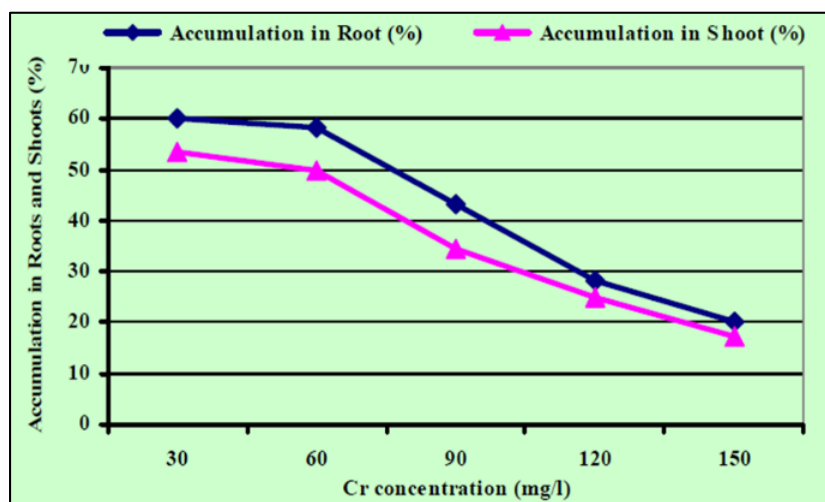


Fig 3: Cr accumulation (%) in root and shoot of spinaches oleracea

M. Ghosh and S. P. Singh 2005 [37] in their comparative study found that the order of Cr extraction in five different weeds and two brassica species was *Ipomoea. carnea* > *Datura innoxia* > *Cassia tora* > *Phragmyteskarka* > *Brassica juncea* > *Lantana camara* > *Brassica campestris*. *Phragmyteskarka* how and much greater tolerance to metals than other plants

but the uptake was less. Other than *Lantana camara*, all the tested weeds were better for chromium extraction than the accumulator *Brassica species*. This indicates that weeds can be used in place of *brassica species* and it requires very less cure (fig 4).

Total Cr added in Soil (mg kg ⁻¹)	<i>Brassica campestris</i>	<i>Brassica juncea</i>	<i>Dhaturo innoxia</i>	<i>Ipomoea carnea</i>	<i>Phragmytes karka</i>	<i>Cassia tora</i>	<i>Lantana camara</i>
Control	3.28	3.31	12.32	19.59	11.46	12.45	5.43
5	1.86**	2.89*	8.57**	15.01**	7.66**	7.90**	2.27**
10	1.47**	2.16**	7.24**	11.33**	5.93**	7.30**	1.93**
20	1.36**	1.17**	6.49**	10.50**	7.64**	7.21**	1.76**
50	NG	NG	NG	NG	1.51**	NG	1.09**
100	NG	NG	NG	NG	1.06**	NG	NG
200	NG	NG	NG	NG	0.78**	NG	NG

Significantly different * ($p \leq 0.05$) & ** ($p \leq 0.005$) in comparison to control plant.
 NG = No Growth observed
 n = number of plants

Fig 4: Average dry biomass (g) grown in chromium treated soils (n= 6) on 90th day
 ~ 321 ~

Although, it has been known since the late 1800s that a special category of plants, the so called hyper accumulators can accumulate extraordinary levels of metals, the idea of using these plants for phytoextraction only appeared in the literature in the Zn up to levels that are 100 to 1,000 times of those normally accumulated by plants grown under the same conditions (Baker *et al.*, 2000; Ma *et al.*, 2001; Brooks, 1998)^[66]. A number of these species are members of Brassicaceae, including a species of Arabidopsis, *A. halleri*, which can hyper accumulate Zn in its shoots (Reeves and Backer, 2000)^[66]. Recently, *Sonchus asper* and *Corydalis pterygotata* grown on lead – zinc mining area in China have been identified as heavy metal hyper accumulators (Yanqun *et al.*, 2005)^[93]. Environment Canada has developed a database (PHYTOREM) of 775 plants with capabilities to accumulate or hyper accumulate one or several key metallic elements. Table 2 lists some important hyper accumulators including the recently discovered ones. Despite these properties hyper accumulators are of limited use for large scale applications

because they are often slow growing and attain low biomass. So far only one hyper accumulator species, the Ni hyper accumulator. *Alyssum bertolonii*, has been used for phytoremediation in the field (Chaney *et al.*, 2000; Li *et al.*, 2003)^[18]. *Pteris vittata*, an Arsenic (As) hyper accumulating fern may also show promise for phytoextraction of As. Brake fern, *Pteris vittata*, a fast growing plant is reported to tolerate soils contaminated with arsenic as much as 1500 p.p.m and its fronds concentrate the toxic metal to 22,630 p.p.m in 6 weeks (Ma *et al.*, 2001). However, in the coming years, mining of the genomic sequences from *Arabidopsis thaliana* and rice and availability of new genomic technologies should lead to identification of novel genes important for heavy metal remediation. The relevant genes from these hyper accumulators may then be introduced into higher biomass producing non-accumulators for an improved phyto remediation potential, making it a commercially viable technology.

Table 2: Several metal hyper accumulator species with respective metal accumulated

S.no	Plant species	Metal	References
1	<i>Thlaspi caerulescens</i>	Zn, Cd	Reeves and Brooks (1983) ^[65] ; Baker and Walker (1990) ^[5]
2	<i>Ipomea alpine</i>	Cu	Baker and Walker (1990) ^[5]
3	<i>Sebertia acuminata</i>	Ni	Jaffre <i>et al.</i> , (1976)
4	<i>Haumaniastrum robertii</i>	Co	Brooks (1977)
5	<i>Astragalus racemosus</i>	Se	Beath <i>et al.</i> , (2002) ^[10]
6	<i>Arabidopsis thaliana</i>	Zn, Cu, Pb, Mn, P	Lasat (2002b) ^[47]
7	<i>Brassica oleracea</i>	Cd	Salt <i>et al.</i> , (1995b) ^[73]
8	<i>Hemidesmus indicus</i>	Pb	Chandra Sekhar <i>et al.</i> , (2005) ^[15]
9	<i>Pteris vittata</i>	As	Ma <i>et al.</i> , (2001); Zhang <i>et al.</i> , (2004) ^[94] ; Tu and Ma (2005)
10	<i>Helianthus anus</i>	Cd, Cr, Ni	Turgut <i>et al.</i> , (2004) ^[86]

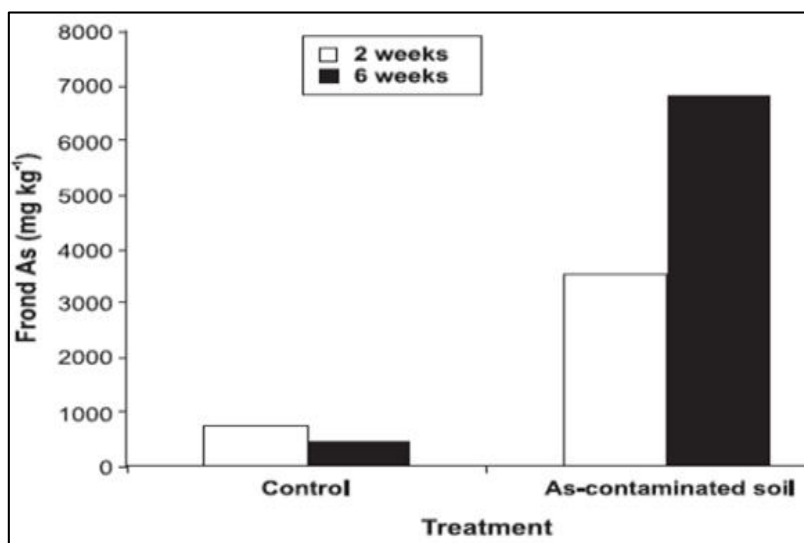


Fig 5: Arsenic (As) concentration in the fronds of *Pteris vittata* after growing in uncontaminated soil (6 ppm As) and Arsenics contaminated soil (400 ppm As)

Role of metal chelators

As mentioned earlier, the complex root secretions from plants contain natural chelating agents that affect pollutant solubility and uptake. Inside plant tissues such chelator's compounds also play a role in tolerance, sequestration and transport (Ross, 1994). Phytosiderophores are chelators that facilitate uptake of Fe and perhaps other metals in grasses (Higuchi *et al.*, 1999)^[41]. Organic acids (e.g. citrate, malate, acetate) not only can facilitate uptake of metals with roots but also play a role in transport, sequestration, and tolerance of metals (Salt *et al.*, 1995b; Von Wiren *et al.*, 1999)^[73, 91]. As a tolerance

and detoxification mechanism, chelated metals are effluxed from cytoplasm and sequestered in the vacuolar compartment, which excludes them from cellular sites where processes such as cell division and respiration occur, thus providing an effective protective mechanism (Chaney *et al.*, 1997; Hall, 2002)^[17, 39]. Detoxification of Cd and Zn in *Thlaspi caerulescens* is achieved by vacuolar compartmentalization (Ma *et al.*, 2005).

Phytoremediation using trees

Trees have been suggested as a low-cost, sustainable and ecologically sound solution to the remediation of heavy metal-contaminated land (Dickinson., 2000) [23], especially when it is uneconomic to use other treatments or there is no time pressure on the reuse of the land (Riddell-Black, 1994) [66]. Studies of tree establishment on contaminated land have considered a number of different species, e.g. *Salix* (*Willow*), *Betula* (*Birch*), *Populus* (*Poplar*), *Alnus* (*Alder*) and *Acer* (*Sycamore*). While many of these studies were interested primarily in metal uptake, distribution within the plant and tolerance mechanisms, for the purposes of phytoremediation, most attention has been paid to fast growing species, such as willow. The genus *Salix* is a member of the Salicaceae plant family. There are 400 species of willow, with more than 200 listed hybrids (Newsholme, 1992) [58]. The majority of the genus *Salix* grow in lowland wetland habitats and have evolved a number of varieties and hybrids (Sommerville., 1992) [79]. A characteristic of willow, which makes it a very suitable tree for use in phytoremediation, is that it can be frequently harvested by coppicing, yielding as much as 10–15 dry $\text{tha}^{-1} \text{year}^{-1}$ (Riddell-Black., 1993) [66].

Bushy *Salix* species with erect stems, rapid growth and good rooting ability are the most suitable for biomass coppice, with *S. viminalis* being one of the most widely used species (Ahman and Larsson, 1994) [1]. In addition to high biomass productivity, *Salix* trees also have an effective nutrient uptake, high evapotranspiration rate and a pronounced clone specific capacity for heavy metal uptake. Possible end-product uses of *Salix* biomass include fuel for direct burning as wood chips, raw material for the production of paper, chipboard and charcoal, a source of viscose for the textile industry, basket weaving and the production of briquettes, ethanol and ruminant livestock feed supplement (McElroy and Dawson, 1986). Use as wood fuel could allow possible heavy metal recovery through the scrubbing of smoke gases and proper handling of ashes (Perttu and Kowalik, 1997; Dahl, 2000) [59].

Transgenic plants in phytoremediation

The plant species currently being developed for phytoremediation seem capable of effective bioaccumulation of targeted contaminant, but efficiency might be improved through the use of transgenic (genetically engineered) plants. Naturally occurring plant species that can be genetically engineered for improved phytoremediation include *Brassica juncea* for phytoremediation of heavy metals from soil (Dushenkov *et al.*, 1995) [28], *Helianthus annuus* (Dushenkov *et al.*, 1995) [28] and *Chenopodium amaranticolor* (Eapen *et al.*, 2003) [29] for rhizofiltration of uranium. In general, any dicotyledon plant species can be genetically engineered using the *Agrobacterium* vector system, while most monocotyledon plants can be transformed using particle gun or electroporation techniques.

The increase in metal accumulation as the result of these genetic engineering approaches is typically two to threefold more metal per plant, which potentially enhances phytoremediation efficiency by the same factor. It is not yet clear how applicable these transgenic are for environmental clean up, since no field studies have been reported except one using transgenic Indian mustard plant that overexpresses enzymes involved in sulfate/selenate reduction. (Pilon Smits *et al.*, 1999; Zhu *et al.*, 1999) [62, 96]. Potential environmental impacts of transgenics such as competitiveness of transgenic to wild type, effect on birds, insects, etc., that might feed on

plant biomass containing high concentration of toxic metals and possibility of gene transfer to other plants by pollination require continuous monitoring. Genetic engineering of the chloroplast genome offers a novel way to obtain high expression without the risk of spreading the transgene via pollen (Ruiz *et al.*, 2003) [70]. In future, as more data on field trials and associated risk assessment would be available, transgenics will play an important role in commercial phytoremediation.

Conclusions

Phytoremediation is still in its research and development phase, with many technical issues needing to be addressed. The results, though encouraging, suggest that further development is needed. Phytoremediation is an interdisciplinary technology that can benefit from many different approaches. Results already obtained have indicated that some plants can be effective in toxic metal remediation. The processes that affect metal availability, metal uptake, translocation, chelation, degradation, and volatilization need to be investigated in detail. Better knowledge of these biochemical mechanisms may lead to: (1) Identification of novel genes and the subsequent development of transgenic plants with superior remediation capacities; (2) Better understanding of the ecological interactions involved (e.g. plant-microbe interactions); (3) Appreciation of the effect of the remediation process on ecological interactions; and (4) Knowledge of the entry and movement of the pollutant in the ecosystem. In addition to being desirable from a fundamental biological perspective, findings will help improve risk assessment during the design of remediation plans, as well as alleviation of risks associated with the remediation. It is important that public awareness of this technology be considered, with clear and precise information made available to the general public to enhance its acceptability as a global sustainable technology. So far, most phytoremediation experiments have taken place on a laboratory scale, with plants grown in hydroponic settings fed heavy metal diets. Both agronomic management practices and plant genetic abilities need to be optimized to develop commercially useful practice.

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